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# THE DISTRIBUTION AND LIFE HISTORY OF *ARRHOPALITES CAECUS* (TULLBERG): ORDER: COLLEMBOLA, IN WIND CAVE, SOUTH DAKOTA, USA

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*Individuals of the collembolan species* Arrhopalites caecus *(Tullberg) were collected from drip pools within Wind Cave, South Dakota, at Methodist Church adjacent to the Natural Entrance Tour Route and Room Draculum near survey marker NP-39. Specimens were identified as* A. caecus *using direct interference and scanning electron microscopy. Molecular analysis of the D2 region of 28S rDNA was performed and the sequences were deposited in Genbank (accession number AY239037). We determined that our population of* A. caecus *reproduced parthenogenetically by successively isolating and rearing eggs through the F4 generation on 9:5 plaster:charcoal media maintained at 21°C, and by the absence of males. Molecular analysis of 16S rDNA for bacteria within our specimens failed to detect the α-proteobacterium (Rickettseales)* Wolbachia*. Generation times, fecundity, and molt frequency were consistent with other reports for Collembola.*

## INTRODUCTION

*Arrhopalites caecus* (Tullberg) was initially described and studied by Tullberg (1871) and Böerner (1906) and has been found in caves and surface localities in Europe and North America (Christiansen, 1966). The genus *Arrhopalites* is one of the more widespread cave forms of Collembola within the Holarctic region (Vandel, 1965). Although a recent catalog of the genus *Arrhopalites* in North American caves (Zeppelini and Christiansen, 2003) did not include caves of the Black Hills of South Dakota, the genus has been collected in both Wind Cave and Jewel Cave. Christiansen and Bellinger (1980, 1998) reported finding *Arrhopalites caecus* in Custer County, South Dakota (the county where Wind Cave is located), while Moore *et al.* (1996) found the species in both Wind Cave and nearby Jewel Cave.

Reports on the geographic distribution of and morphological description of *A. caecus* are available, but little information on its behavior, and to our knowledge, no information on the D2 region of its 28s rDNA or its life history have been reported. Given the ease that we were able to initiate viable cultures from single individuals, we suspected that *A. caecus* might reproduce parthenogenetically. The objectives of our study were as follows: 1) determine the nucleotide sequence of the D2 region of the 28s rDNA of *A. caecus*, 2) determine the extent of the distribution of *A. caecus* within Wind Cave, and 3) observe and document the life history of *A. caecus*.

## SITE DESCRIPTION

Wind Cave is located in Wind Cave National Park on the southeastern flank of the Black Hills of South Dakota, near the town of Hot Springs (Fig. 1). Wind Cave is a phreatic dissolution cave in the Madison Limestone and overlying Minnelusa Formation, that formed during the uplift of the Black Hills between 65 and 40 million years ago and continued to be modified during the erosion that has occurred over the past 40 million years. The Madison Limestone was deposited over 330 million years ago during the life and evaporation of a warm shallow sea, while the Minnelusa Formation, consisting of sandstone, shales, and carbonates, was deposited 300 million years ago during a subsequent advance and retreat of a later sea (Palmer and Palmer, 2000). Wind Cave is a network maze cave with five recognized levels and up to 76 meters of vertical relief at any given point. The cave only has two natural entrances and is fairly well sealed by the overlying Minnelusa Formation. The cave is dry, except where it lies underneath modern drainages or where it hits the water table at its deepest point (Horrocks and Szukalski, 2002). The upper levels of the cave started draining 350,000 years ago and were completely air-filled by 155,000 years ago (Ford, 1993). Surface vegetation of the landscape overlying Wind Cave has been described as northern mixed grass-prairie (80%) with woodlands (20%) dominated by *Pinus ponderosa* Dougl. (Coppock *et al.,* 1983; Whicker and Detling, 1988).

## METHODS

### SURVEYS OF SOILS AND SEDIMENTS

We determined the distribution of *A. caecus* within Wind Cave using a combination of strategies including the systematic and opportunistic sampling of soils, sediments and water surfaces, and sampling with pitfall traps conducted from June 1992 through October 2004. For the systematic sampling of the cave a 152.4 x 152.4 m (actual dimension was 500 x 500 ft) grid was positioned over a map of the cave, and sediments

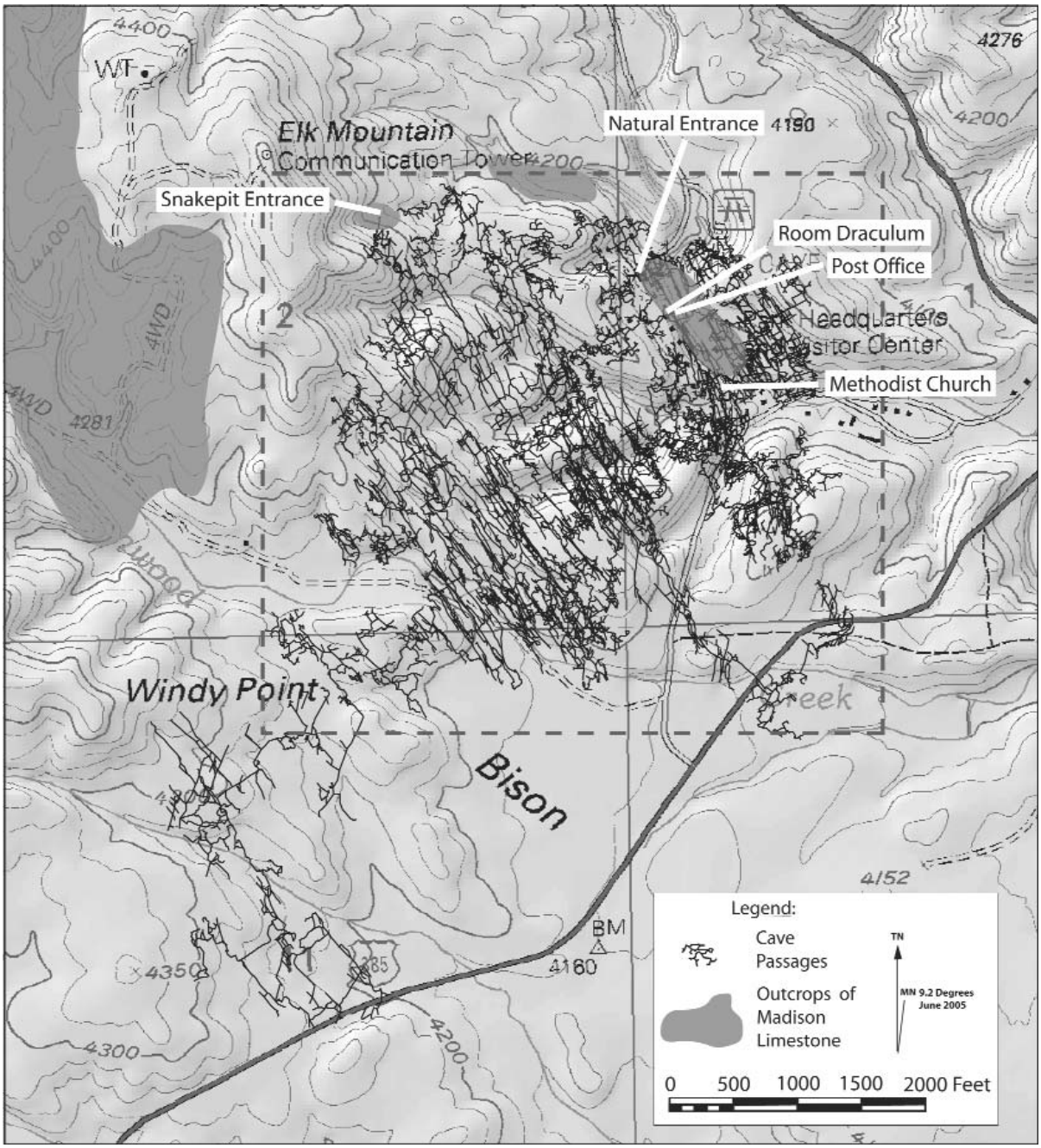


**Figure 1. Topographic map with an overlay of the current known extent of Wind Cave (adapted from Horrocks and Szukalski, 2002). The box framed with dashed lines approximates the extent of the 1992–1996 survey, where sampling occurred at 152.4 x 152.4 m grid intersections. The Natural Entrance and Snake Pit are known entry points into the cave.**

were sampled at locations closest to the grid intersections. For the systematic sampling of surface soils, three 5 cm diameter x 20 cm deep cores were taken at 10 m intervals along each of three 100 m transects spaced 200 m from one another, perpendicular in orientation to Wind Cave Canyon. Collembola were extracted from the soils using modified Tullgren funnels (Moore *et al.*, 2000) and identified using the keys of Christiansen and Bellinger (1998) and Janssens (2003). Opportunistic sampling from different locations occurred on survey trips or in conjunction with other studies. Sampling included pitfall traps lined with a 9:5 plaster charcoal, open Petri dishes lined with 2% water agar, collection of specimens from the surface of drip pools using brushes and aspirators, and extraction from sediments, as described above.

### COLLECTION AND REARING

We selected four individuals from laboratory colonies of *A. caecus* established using specimens collected from pools at Methodist Church adjacent to the tour path on the Natural Entrance Tour Route and Room Draculum near marker NP-39. Each individual was placed in a fresh vial containing a medium of plaster and charcoal in a 9:5 ratio (Snider *et al.,* 1969) and fed baker's yeast. All vials were kept moist and incubated at 21° C and observed daily. There were a few 1-day and 2-day interruptions throughout the study, and one 4-day interruption for the F2 generation. Beginning with the parental generation (P), as eggs were laid, each egg was separated into its own fresh vial containing the 9:5 ratio plaster-charcoal medium.

The date that the egg was laid and identification of the parent were recorded. When an egg hatched, the date was recorded and a grain of baker's yeast added. Individuals were observed daily (with a few interruptions as noted above), with the date of each of its molts and its death recorded. When subsequent generations began to lay eggs, the same process of separating eggs and recording molts and death was followed until laboratory supplies were exhausted. Excluding the four parents, 90 individuals across four generations were studied.

### IDENTIFICATION

Specimens from the field and laboratory cultures were identified using light microscopy, scanning electron microscopy, and molecular phylogenetic methods using 28S rDNA. For identification with light microscopy, adults were selected from the cultures, killed in 70% ethanol, and cleared in dilute Hoyer's solution. Cleared specimens were mounted in Hoyer's solution and observed under an Olympus BH-2 outfitted with Direct Interference Contrast. For scanning electron microscopy, specimens were killed and dehydrated using hexamethyldisilazane (Nation, 1983). Specimens were mounted and sputter-coated with 15 μm of gold and examined using a Joel JSM-5200 scanning electron microscope.

For molecular analysis and identification, DNA was extracted and purified using a commercial DNA extraction kit (Mo Bio Laboratories, CA). The D2 region of 28S rDNA was PCR-amplified using primers C2 (5'-GAAAA-GAACTTTGRARAGAGAGT-3') (Friedrich and Tautz, 1997) and D2Coll (5'- ACCACGCATGCWTTAGATTG- 3') (D'Haese 2002). Purified DNA was used as a template in 50 μl PCR reaction containing 1X Taq buffer (Eppendorf), 2 mM $Mg(Oac)_2$, 0.5X TaqMaster PCR Enhancer (Eppendorf), 200 μM dNTPs, 20 pmol of each primer, and 0.5 U Taq polymerase. Touchdown PCR was used with a 3 minute denaturation at 95°C followed by 30 cycles at 94°C for 15 seconds, annealing for 15 seconds, and extension at 72°C for 20 seconds, with a final extension at 72°C for 7 minutes. Annealing began at 62°C and decreased 0.5°C each successive cycle until 58°C was achieved for the remaining 20 cycles. The PCR product was purified using the Micron PCR purification kit (Millipore, MA). The ABI cycle sequencing kit was used by the University of Colorado Cancer Center Sequencing and Analysis Core to sequence the entire PCR product using primers C2 and D2 Coll.

### AMPLIFICATION OF ENDOSYMBIONT 16S RDNA

We attempted PCR amplification of the α-proteobacterium *Wolbachia* from individual *Arrhopalites caecus* (Tullberg) and *Folsomia candida* (Willem) using the methods described above. We targeted our PCR-amplification to 16S rDNA using the primers ftsZunif, ftsZunir (Lo *et al.* 2002), ftsZf1, ftsZr1, ftsZAdf, ftsZAdr, ftsZ1, ftsZ2 (Werren *et al.*, 1995), and ftsZcolr (Czarnetzki and Tebbe, 2004) specific to *ftsZ*, a cell division gene; primers wspf and wspr (Braig *et al*., 1998), specific to *wsp* which encodes a cell surface protein, and primers 16SAf, 16SAr (Werren *et al*., 1995) 27f, 1492r (Lane, 1991), specific to 16S rDNA. Because these amplifications failed, double PCR was employed using primer pairs ftsZunif and ftsZunir, ftsZf1 and ftszr1. In this case, one μL of PCR product from the initial round of amplification was used as template in the subsequent PCR containing the same primer pairs used in the first round of amplification. Additionally, double PCR using internal primers (ftsZunif and ftsZunir, ftsZ1 and ftsZunir, ftsZ1and ftsZcolr) was used after an initial amplification with ftsZunif and ftsZcolr and the internal primers ftsZunif and ftsZunir were used after amplification with ftsZf1 and ftsZr1.

## RESULTS

### IDENTIFICATION

We identified adult specimens collected from the field and from our laboratory cultures as *Arrhopalites caecus* using the keys developed by Christiansen and Bellinger (1998) and Janssens (2003). Adult *A. caecus* possessed a reddish-brown pigmentation across their dorsal surface and a bleached variation of this coloration along their ventral surface. Specimens possessed a pair of antennae that were longer than the width of the head (Fig. 2A). The fourth antennal segment was longer than the third antennal segment and possesses faint evidence of subdivision (Fig. 2A). The reduced eyes were 1+1 (Fig. 2B). Spine-like medial cephalic setae were present (Fig. 2C). The ventral surface of the dentes possessed 3 proximal setae ($Ve_3$, $Ve_4$, and $Ve_5$), with the medial $Ve_1$ setae being spine-like (Fig. 2D). The dorsal surface of the dentes possessed proximal $e_6$ setae (Fig. 2D). The unguis of the second leg appeared to possess a weak tunica (Fig. 2E). The anal valve possessed spines (Fig. 2F).

At the time of identification, no *A. caecus* molecular sequences had been deposited in Genbank. The D2 region of 28S rDNA (397 bases) was sequenced and deposited in Genbank with the accession number AY239037. The closest relative in the database was *A. sericus*, sharing 84% sequence identity with *A. caecus*.

### DISTRIBUTION

*A. caecus* was collected from only two of the over 50 sites that were sampled in Wind Cave from 1992-2004 (see Fig. 1). Specimens were sighted and collected at several locations within the Room Draculum and along the Natural Entrance Tour Route in a drip pool at the Methodist Church (Fig. 3). Room Draculum is located between 18–22 m below the floor of Wind Cave Canyon and is found in the Upper Middle Level of Wind Cave. The presence of stream-rounded cobbles in this room indicates that the area may have possessed an entrance at some point in the distant past. Cobbles found at the potential entrance appear to match Tertiary-aged cobbles found on abandoned terraces within Wind Cave National Park. Methodist Church is located 44–49 m below that same drainage and is located in the Middle Level of Wind Cave. These sites are sep-

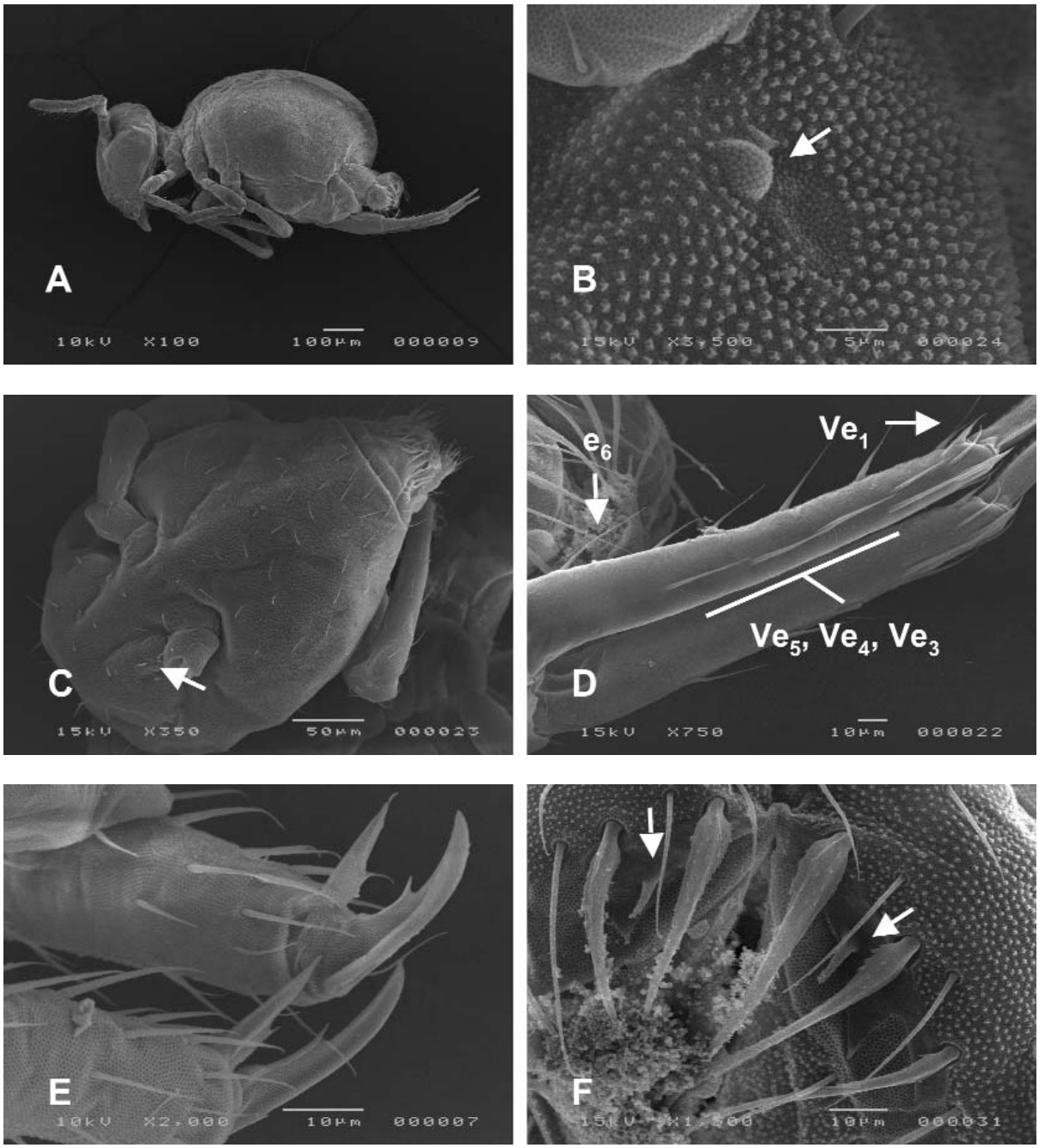


**Figure 2. Scanning electron micrographs of morphological features used to identify *A. caecus*. A) Lateral view of an adult *A. caecus*, fourth antennal segment more than half as long as third, antennal segment longer than head; B) Single ocellus (arrow) and adjacent post antennal organ (depression); C) Spinelike dorsal cephalic setae; D) Ve setae complex the dens, ventral surface of dens possesses 3 proximal setae; E) Tarsal setae, absence of clavate tenate hairs; F) pair of anal spines (arrows).**

arated horizontally by 107 m and vertically by 27 m. No specimens were found in soil samples taken outside the cave during surface soil surveys.

### LIFE HISTORY

Eggs were round to slightly oblong and measured 0.154 ± 0.013 mm (n = 8) at the long ends and were white with a rough surface. As is typical for Collembola the eggs darkened with age. Gestation averaged 22.2 ± 5.2 days, ranging from 16.2 ± 6.3 days for the F1 generation and to 28.8 ± 5.6 days for the F4 generation (Table 1). Juveniles were white for the first two instars, and darkened after subsequent molts to a tan to rust color. Instar duration ranged from 5.2 ± 2.7 to 20 ± 2.8 days, with the first and second instars being longer than subsequent instars (Table 1).

The majority of individuals matured to adults during the second instar. Of the individuals that hatched from eggs laid during the study, eight individuals produced their first eggs within the first instar, and 50 individuals produced their first eggs within the second instar (Fig. 4). Our assessment of 381 egg laying events found that the average clutch size was 3.5 ± 0.4 eggs, with individuals producing between 1 and 18 eggs with an average of 1.6 ± 0.3 events per instar (Table 2). Fecundity of successive generations declined with reduced numbers of events per instar, and maximum and mean clutch sizes (Table 2).

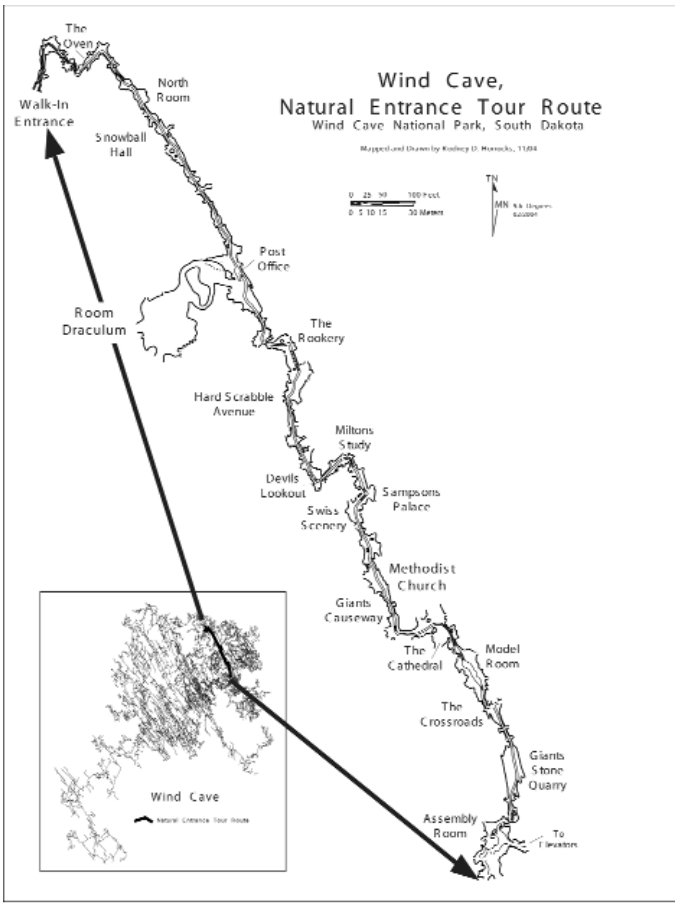


**Figure 3. Populations of *Arrhopalites caecus* were discovered in Room Draculum and Methodist Church, and reported in the Post Office (K. Christensen *pers. comm.* 2003).**

Figure 5 presents a survivorship curve and timeline constructed from averages across the F1 through F4 generations. For a study cohort of 128 eggs for which we had complete information, 78% hatched and 51% matured to adulthood (2nd Instar). The survivorship curve drops precipitously after the 2nd instar with fewer than 5% surviving to the 5th instar. A single individual from the F2 generation survived 10 instars (died during the 11th instar).

Our population of *A. caecus* reproduced parthenogenetically. Isolated eggs collected and reared to adults from the parental through the F3 generation produced viable offspring, i.e., F4 offspring and eggs (F5 generation) were produced. No males were found in the samples collected from the field or within our laboratory cultures.

### THE ENDOSYMBIONT *WOLBACHIA*

We were unable to amplify α-proteobacterium *Wolbachia* from *A. caecus* but were able to amplify *Wolbachia* from *F. candida* that we had also collected from Wind Cave (Fig. 6). *FtsZ* and 16S rDNA primers that have PCR amplified *Wolbachia* within *F. candida* (Czarnetzki and Tebbe, 2004; Lo

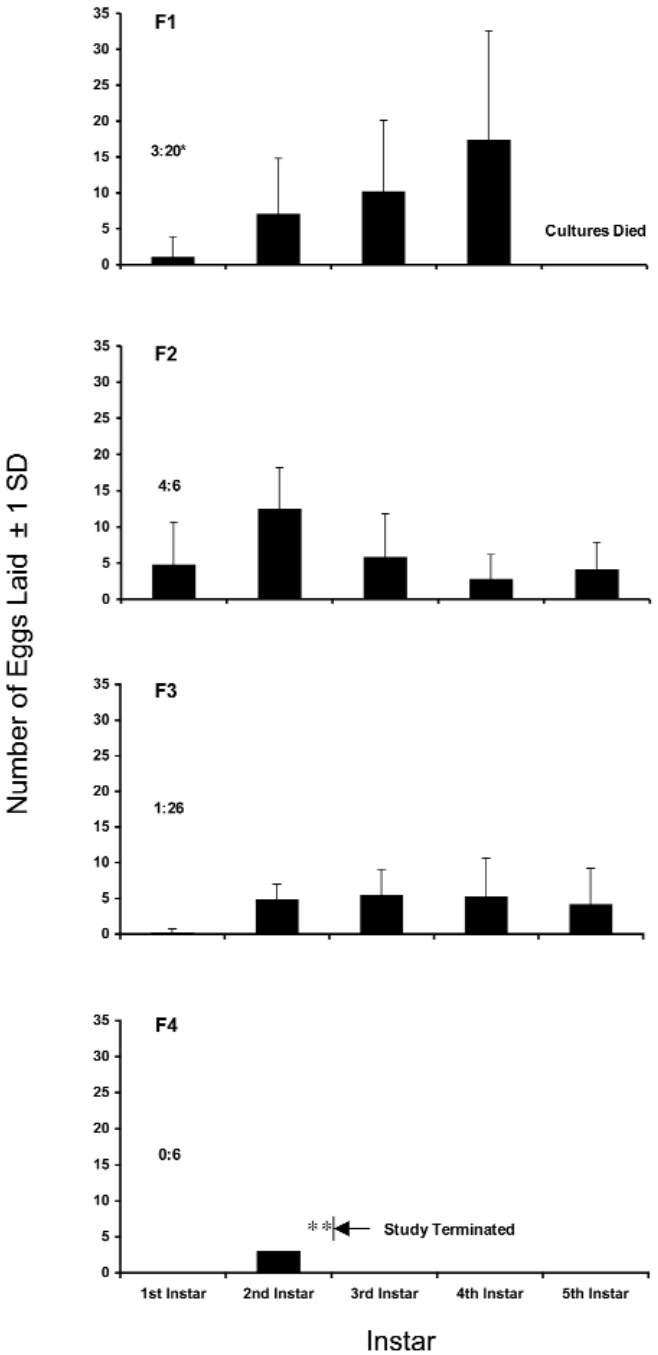


**Figure 4. Fecundity of the F1 through F4 generations of *A. caecus*, as indexed by egg production (number of eggs ± S.E.) by instar. The ratio appearing above the bars for the 1st instar represents the number of individuals that matured in the 1st instar: total number of individuals that matured. The instar in which an individual matured was marked by the appearance of eggs.**

*et al.,* 2002) and the sister group A members (Hong *et al.*, 2002; Werren *et al*., 1995) failed to generate a PCR product with *A. caecus.* Given that initial attempts to PCR amplify *Wolbachia* associating with *Folsomia* (Lo *et al.,* 2002) and

**Table 1. Egg development times (days ± S.D.) and instar durations (days ± S.D.) for the F1 through F4 generations. The number of eggs or individuals is in parentheses.**

| Generation | Egg Development Time | Instar Duration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st Instar | 2nd Instar | 3rd Instar | 4th Instar | 5th Instar | 6th Instar | 7th Instar | 8th Instar | 9th Instar |
| F1 | 16.2 ± 6.3 (25) | 10.2 ± 8.1 (20) | 11.4 ± 9.1 (14) | 5.4 ± 5.4 (7) | 8.5 ± 6.4 (2) | N.A.[a] | N.A. | N.A. | N.A. | N.A. |
| F2 | 22.6 ± 8.1 (15) | 20.0 ± 2.8 (6) | 16.9 ± 1.9 (7) | 8.2 ± 0.8 (5) | 12.2 ± 2.5 (5) | 5.2 ± 2.7 (5) | 12.0 ± 3.5 (4) | 7.7 ± 0.7 (2) | 9.5 ± 3.5 (2) | 9.0 ± N.A (1) |
| F3 | 21.1 ± 9.4 (25) | 14.0 ± 1.6 (26) | 15.5 ± 1.5 (24) | 7.9 ± 2.0 (19) | 10.3 ± 2.4 (18) | 9.1 ± 1.8 (11) | N.A. | N.A. | N.A. | N.A. |
| F4 | 28.8 ± 5.6 (25) | 13.0 ± 1.6 (6) | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| Aggregated Generations | 22.2 ± 5.2 (4) | 14.3 ± 4.1 (4) | 14.6 ± 2.8 (3) | 7.2 ± 1.5 (3) | 10.3 ± 1.9 (3) | 7.1 ± 2.8 (2) | 12.0 ± 3.5[b] (1) | 7.6 ± 0.7[b] (1) | 9.5 ± 3.5[b] (1) | 9.0 ± N.A. (1) |

[a] N.A.= no data available, all the organisms within the generation died, or a single organism remained.
[b] Mean and standard deviation of the F2 generation are reported, as no data are available for instars 6 – 9 for the F1, F3, and F4 generations.

**Table 2. The mean number of egg laying events (events ± S.D.) and clutch sizes (number eggs ± S.D.) for instars > 2 for the F1 through F4 generations. The number of events or eggs is in parentheses.**

| Generation | Events >2nd Instar | Clutch Size | | |
|---|---|---|---|---|
| | | Individual | Pooled | Maximum |
| F1 | 2.0 ± 0.8 (4) | 4.3 ± 1.4 (21) | 4.1 ± 3.4 (143) | 18 (143) |
| F2 | 1.5 ± 0.7 (10) | 3.7 ± 0.5 (16) | 3.6 ± 2.3 (126) | 13 (126) |
| F3 | 1.4 ± 0.4 (5) | 3.4 ± 1.0 (26) | 3.5 ± 2.1 (121) | 12 (121) |
| F4 | N.A. | 3.0 ± N.A. (1) | 3.0 ± N.A. (1) | 1 (1) |
| Grand Mean ± S.D. | 1.6 ± 0.3 (3) | 3.6 ± 0.6 (4) | 3.5 ± 0.4 (4) | 18 (381) |

unrelated hosts (Jeyaprahash and Hoy, 2000) have produced false-negative results, and the parthenogenetic life history of *A. caecus*, it is possible that *Wolbachia* is present within *A. caecus* but is intractable using the methods described here. Therefore, we are pursuing the use of universal 16S rDNA PCR primers in conjunction with cloning and sequencing to reveal all bacteria associating with *A. caecus.*

## DISCUSSION

Our specimens keyed directly to *Arrhopalites caecus* using the keys of Christiansen and Bellinger (1998) for Nearctic *Arrhopalites*. Spine-like medial cephalic setae are clearly visible, as is the full complement of the proximal ventral $Ve_1$-$Ve_3$ setae. The key provided by Janssens (2003), which focuses on *Arrhopalites* worldwide, failed at the final couplet to definitely distinguish our specimens as either *A. caecus* or *A. minutus,* given our conservative interpretation of the more subjective characteristics. Consistent with *A. caecus* our specimens possessed dorsal dental seta $e_6$. However, consistent with *A. minutus*, the fourth antennal segment is arguably not subdivided and the second unguis does not possess a pronounced tunica, i.e., arguably absent. Moreover, consistent with *A. minutus*, the anal valves possess a single spine rather than a pair of spines. Christiansen and Bellinger (1998) note that for Nearctic species, the absence of a subdivided fourth antennae segment may occur for *A. caecus*, and the presence of anal spines was a useful but not a definitive diagnostic character for the species.

Previous surveys within Wind Cave have provided conflicting reports on the distribution of *Arrhopalites*. Peck (1959) surveyed Wind Cave and did not report *Arrhopalites* but did report, albeit tentatively, a closely related genus *Pararrhopalites*. We suspect that this earlier survey may have based the identification on a juvenile of *A. caecus*. Our finding *A. caecus* in Room Draculum and at Methodist Church in Wind Cave confirms the earlier report by Christiansen (1966). Though the location within Wind Cave was not disclosed in the 1966 paper, Christiansen communicated finding *A. caecus* near the Post Office room (Christiansen, personal communication, 2003). If we combine the collection at the Post Office

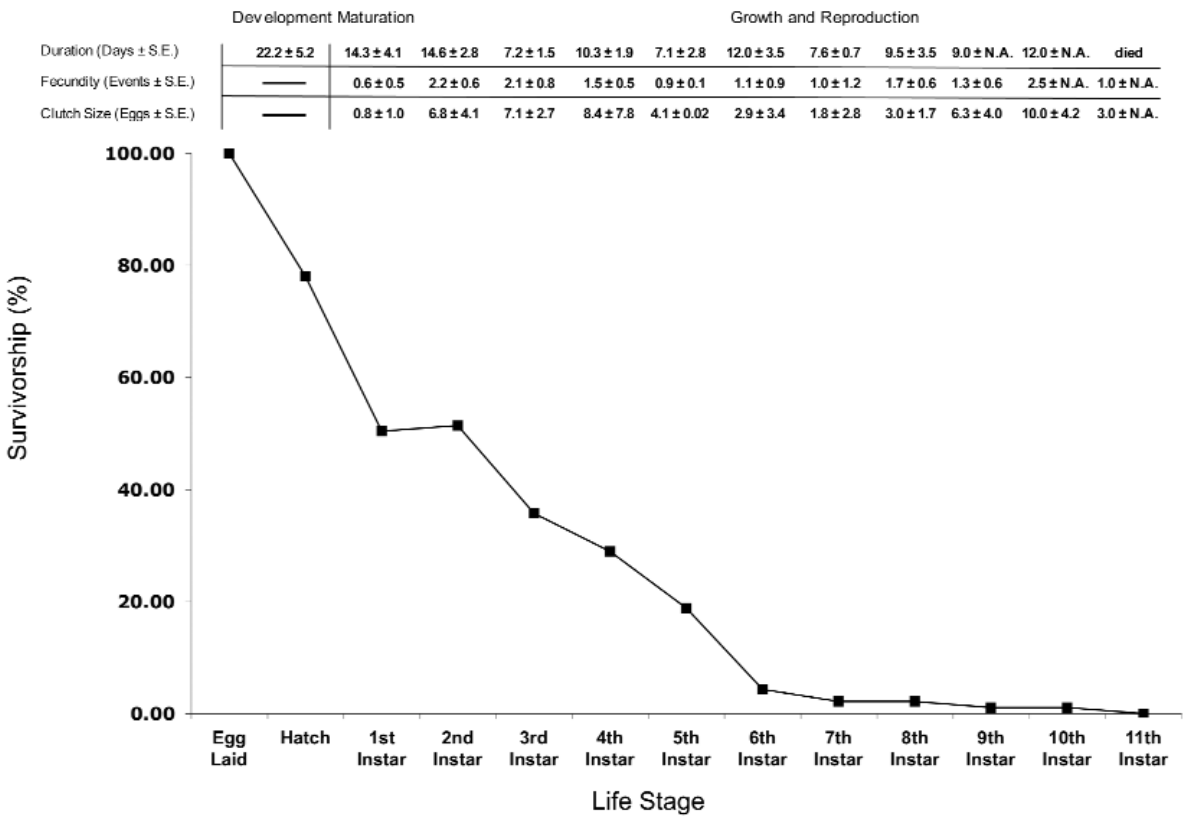


**Figure 5. Survivorship curve generated from the compilation of the unweighted averages of data across instars. The time line presented above the survivorship curve includes the generation means presented in Table 1.**

with our collections at Methodist Church and Room Draculum, the distribution of *A. caecus* within Wind Cave is localized since the Post Office is the jump point to Room Draculum and is along the same tour route as Methodist Church (Fig. 1, Fig. 3). Moreover, all sites possess or are near drip pools that originate from the above ground drainage of Wind Cave Canyon. These sites represent a fraction of the soils and drip pools beneath other drainages throughout Wind Cave (Horrocks and Szukalski, 2002).

Several hypotheses could explain why *A. caecus* was collected in a restricted region of the cave and appears not to have expanded further within Wind Cave. Three possibilities include limitations in the amount of available energy, a low colonization rate, and the compatibility of sediments. The latter two explanations are unlikely for several reasons. *A. caecus* co-occurs with the collembolans *Folsomia candida* (Willem) and three species in the family Entomobryidae and with a campodied dipluran (Moore *et al.*, 1996). Given the age of the cave and the vagile nature of the animal, *A. ceacus* has had ample opportunity to colonize even the more remote sections of the cave, as did the other species. The dearth of energy inputs into the cave is the more likely explanation. Previous studies in Wind Cave demonstrated a precipitous collapse of the trophic structure correlating with energy inputs (Moore *et al.,* 1996; Jesser, 1998; Moore and de Ruiter, 2000) in a manner consistent with current theory (Oksanen *et al.,* 1981; Moore *et al.,* 2003). The Methodist Church site received higher rates of energy input in the form of human skin cells, hair and clothing lint than Room Draculum owing its proximity to the tour route. Neither site supported predators of Collembola. Other sites receiving lower rates of energy inputs supported only bacteria, fungi and Protozoa. Similar relationships between the occurrence and abundance of collembola and energy inputs have been reported (Christiansen, 1961; Christiansen *et al.*, 1961). In their study of Hunters Cave in the Galena Limestone of the Kansan Drift in northeastern Iowa, Christiansen *et al.* (1961) reported that densities of *A. caecus* and species of *Megalothorax*, *Isotoma*, *Tullbergia*, *Tomocerus*, *Oncopodura*, and *Onychiurus* were strongly correlated with the organic matter content of the substrate, and weakly correlated with the particle size of the substrate.

Samples taken outside the Natural Entrance and from soils along the Wind Cave Canyon drainage did not yield any *A. caecus*. The only other reports of *Arrhopalites* in the Black Hills of South Dakota that we could find were from Jewel Cave, where the senior author reported collecting *A. caecus* at a single site – a pool formed atop a boulder adjacent to the tour route in the Tape Room (Moore *et al*., 1996). An earlier survey by Olson (1977) reported finding *F. candida* and *Entomobrya troglobita* within Jewel Cave, but no record of *A. caecus.*

There have been many publications on the taxonomic classifications and geographic distribution of *Arrhopalites*, but few detailed accounts of life history. This study represents the first comprehensive study of the life history of *Arrhopalites caecus*. The specifics of the life history are not new for Collembola as a whole, but do in some instances represent extremes for the

group or new findings for the genus *Arrhopalites* (Hopkin, 1997). Average clutch size and frequency of egg laying were similar to other Collembola (Snider, 1973; Hopkin, 1997). Embryonic development for *A. caecus* was considerably longer at 22.2 ± 5.2 days than that of other species, which ranged from 6–11 days, when reared at similar temperatures (Snider, 1973; Von Allmen and Zettel, 1983; van Straalen and Joose, 1985). The relatively high mortality rate for eggs (~22%) was due largely to fungal contamination, as many of the eggs that did not hatch were covered with a dense mat of fungal hyphae. The frequency of molts and instar durations are comparable to several species (Hopkin, 1997; Snider, 1973; Krool and Bauer, 1987). The decline in fecundity that we observed with successive generations was likely due to an inadequacy in the yeast as the sole source of the diet (Fig. 4).

Our results suggest that *A. caecus* reaches sexual maturity during the 1st Instar, as eight of the 58 hatchlings produced eggs prior to molting. However, these results are misleading, as our records suggest that six of the eight early maturing individuals may have indeed molted to the 2nd instar but were not recorded. Four of the eight early maturing individuals occurred in the F2 generation, where we had a 4-day gap in data collection coincident with when the first molt might have occurred based on our observations from the F1, F3 and F4 generations (see discrepancy in Table 2). Our laboratory notes indicated that two of the three early maturing individuals from the F1 generation had grown significantly and developed pigmentation before a molt was recorded, indicating that molts had occurred but went unnoticed. Nonetheless, maturation during the second instar is early. Hopkin (1997) reported only 1 species, *Mesaphorura krausberi* (*sensu* Hale 1965) reaching sexual maturity at this early instar, with most species reaching maturity between instar five and eight.

We concluded that *A. caecus* exhibited parthenogenetic reproduction using the conservative criterion of Hopkin (1997), which stipulated the appearance of F4 offspring from the isolated eggs of successive generations. We suspect *A. caecus* is also capable of sexual reproduction, but could not draw this conclusion from our study as we did not encounter any males or observe any spermatophore-like structures in our laboratory cultures. Parthenogenesis may help explain the wide global distribution of *Arrhopalites*, the rarity of encountering males (Christiansen and Bellinger, 1996), as well as historic difficulties in collection (Zeppelini and Christiansen, 2003).

Parthenogenicity offers several selective advantages to populations in cave environments, particularly those with extremely low energy inputs that are patchily distributed throughout the cave. Parthenogenetic reproduction is common among euedaphic Collembola living in stable environments (Hopkin, 1997). Caves offer stable environments, but the dearth of energy poses additional pressures as successful colonization of a site requires either the establishment of gravid females that successfully hatch both males and females, or the successful establishment of both males and females. Parthenogenetic reproduction allows a colony to be established

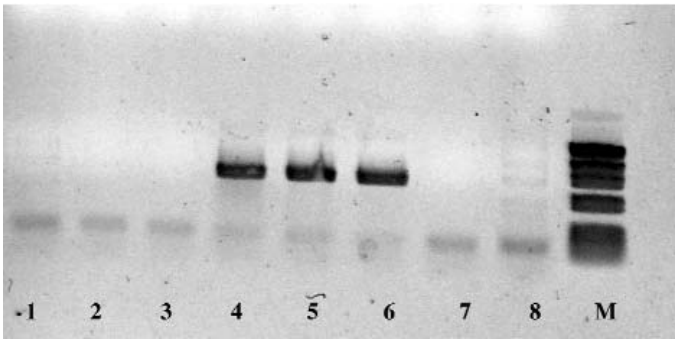


**Figure 6. PCR amplification of *Arrhopalites caecus* and *Folsomia candida* using a gradient of annealing temperatures with primers ftsZunif and ftsZunir (Lo *et al.* 2002). Lanes: 1-3, 8, *Arrhopalites caecus*; 4-6, *Folsomia candida*; 7, negative control; M, marker Phi X174 *Hae* III digest. Lanes: 1,4,7, 50º C anneal; 2, 5, 53º C anneal; 3, 6, 56º C anneal; 8, 48º C anneal.**

by the immigration of a single female. Once established, this colony could retain reproductive viability at very low densities, especially in low temperature and low energy-input caves, making the likelihood of encountering an individual low.

While we were able to confirm that the endosymbiotic *Wolbachia*, an α-proteobacterium (Rickettseales) is present within *F. candida* (Fig. 6) isolated from Wind Cave, we could find no evidence of *Wolbachia* in *A. caecus*. Our search included using primers to detect the protein-coding *FtsZ* gene and 16S rRNA gene primers that have PCR amplified *Wolbachia* within *F. candida. Wolbachia* is maternally transferred, infecting the gonadal tissues. Vandekerckhove *et al.* (1999) noted that the bacterium has been shown to enhance host fertility (Hoerauf *et al.,* 1999) and lead to reproductive isolation of the host population by causing cytoplasmic incompatibility (Bourtzis *et al.,* 1996; Breeuwer, 1997) or inducing parthenogenesis by the feminization of males (Juchault and Legrand, 1989; Juchault *et al.*, 1994) or by initiating thelytokous (= mother-to-daughter) parthenogenesis (Stouthamer *et al.,* 1990; Zchori-Fein *et al.*, 1995). To date six subgroups of *Wolbachia* (designated A-F) have been isolated, with subgroups A and B infecting various arthropods, subgroups C and D infecting filarial nematodes, subgroup E infecting arthropleonan Collembola (*F. candida*, *Mesaphorura italica, M. macrocheata*, *Paratulbergia calliphygos*) and subgroup F infecting termites. We could find no reports of *Wolbachia* infections within symphyleonan Collembola like *A. caecus*.

## CONCLUSIONS

The distribution and life history of *A. caecus* in Wind Cave makes sense when viewed from current evolutionary and ecological perspectives. From an evolutionary context, *A. caecus* possesses all the life history traits favored by natural selection; rapid maturation, early and high fecundity, and low mortality.

Moreover, parthenogenesis is common among collembolans that reside in stable environs (Stam *et al.,* 1996). From an ecological standpoint, *A. caecus* occupies sediments within sections of the cave that possess a stable climate with sufficient energy to meet its growth and reproductive needs, but not enough energy to support a stable population of predators (Roberts, 1974; Moore *et al.,* 1993). Issues that are yet to be resolved for *A. caecus* in Wind Cave are its longevity and details of the physiological basis of its parthenogenesis, as we could find no evidence of infection by the α-proteobacterium *Wolbachia*.

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## REFERENCES

Böerner, C., 1906, Das system der Collembolen, nebst Bescheibung neuer Collembolen des Hamberger Naturhistorischen Museums: Mitteilungen aus dem Naturhistorischen Museums Hamburg, v. 23, p. 147–188.

Bourtzis, K., Nirgianaki, A., Markakis, G., and Sacvakis, C., 1996, *Wolbachia* infection and cytoplasmic incompatability in *Drosophila* species: Genetics, v. 144, p. 1063–1073.

Braig, H.R., Zhou, W.-G., Dobson, S.L., and O'Neill, S.L., 1998, Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. Journal of Bacteriology: v. 180, p. 2373–2378.

Breeuwer, J.A.J., 1997, *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestani*: Heredity, v. 79, p. 41–47.

Christiansen, K., 1966, The genus *Arrhopalites* (Collembola: Sminthuridae) in the United States and Canada: International Journal of Speleology, v. 2, p. 43–73.

Christiansen, K., 1961, The Collembola of Hunters Cave: Bulletin of the National Speleological Society, v. 23, p. 59–63.

Christiansen, K., 2003, *Personal Communication* 7/17/2003.

Christiansen, K., and Bellinger, P., 1980, The Collembola of North America, North of the Rio Grande- A taxonomic analysis: Grinnell College, Grinnell Iowa, 1322 p.

Christiansen, K., and Bellinger, P., 1998, The Collembola of North America North of the Rio Grande, A taxonomic analysis, 2nd edition: Grinnell College, Iowa, 1520 p.

Christiansen, K., and Bellinger, P., 1996, Cave *Arrhopalites* new to science: Journal of Cave & Karst Studies, v. 58, p. 168–180.

Christiansen, K., Wilson, M., and Tecklin, J., 1961, Preliminary study of the microarthropod ecology of Hunters Cave: Bulletin of the National Speleological Society, v. 23, p. 63–70.

Coppock, D.L., Detling, J.K., Ellis, J.E., and Dyer, M.I., 1983, Plant-herbivore interactions in a North American mixed grass prairie. I. Effects of black-tailed prairie dogs on intraseasonal plant biomass and nutrient dynamics and plant species diversity: Oecologia, v. 56, p. 1–9.

Czarnetzki, A.B. and Tebbe, C.C., 2004, Detection and phylogenetic analysis of *Wolbachia* in collembola: Environmental Microbiology, v. 6, p. 35–44.

D'Haese, C.A., 2002, Were the first springtails semi-aquatic? A phylogenetic approach by means of 28S rDNA and optimization alignment: Proceedings of the Royal Society of London B, v. 269, p. 1143–1151.

Ford, D.C., 1993, Uranium-series dating of the draining of an aquifer: The example of Wind Cave, Black Hills, South Dakota: Geological Society of America Bulletin, v. 105, p. 241–250.

Fredrich, M., and Tautz, D., 1997, Evolution and phylogeny of Diptera: a molecular phylogenetic analysis using 28S rDNA sequences: Systematic Biology, v. 46, p. 674–698.

Hale, W.G., 1965, Post-embryonic development in some species of Collembola: Pedobiologia, v. 5, p. 228–243.

Hoerauf, A., Nissen, P.K., Schmetz, C., Henkle-Duehrsen, K., Blaxter, M.L., Buetner, D.W. Gallin, M.Y., Al-Qauod, K.M., Lucius, R., and Fleischer, B., 1999, Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility: Journal of Clinical Investigations v. 103, p. 11–18.

Hong, X.-Y., Gotoh, T., and Hiroaki, N., 2002, Sensitivity comparison of PCR primers for detecting *Wolbachia* in spider mites: Journal of Applied Entomology and Zoology, v. 37, p. 379–383.

Horrocks, R.D., and Szukalski, B.W., 2002. Using Geographic Information Systems to develop a cave potential map for Wind Cave, South Dakota: Journal of Cave and Karst Studies, v. 64, p. 63–70.

Hopkin, S., 1997, Biology of the Springtails: Oxford University Press, Oxford, 330 p.

Janssens, F., 2003, Checklist of the Collembola: Key to the species of *Arrhopalites:* http://www.collembola.org/key/arrhopal.htm

Jesser, R., 1998, Effects of productivity on species diversity and trophic structure of detritus-based food webs within sediments of Wind Cave, South Dakota. [Master's Thesis], University of Northern Colorado, Greeley, CO 80639, USA.

Jeyaprakash, A. and Hoy, M.A., 2000, Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species: Insect Molecular Biology, v. 9, p. 393–405.

Juchault, P., and Legrand, J.J., 1989, Sex determination and monogamy in terrestrial isopods *Armadillidium vulgare* (Latreille, 1804) and *Armadillidium nasatum* (Budde-Lund, 1885): Monitore Zoologico Italiano (N.S.) Monografia, v. 4, p. 359–375.

Juchault, P., Frelon, M., Bouchon, D. and Rigaud, T., 1994, New evidence for feminizing bacteria in terrestrial isopods: evolutionary implications: Comptes Rendus de l'Academie des Sciences. Paris, Series III, v. 317, p. 225–230.

Krool, S., and Bauer, T., 1987, Reproduction, development and pheromone secretion in Heteromurus nitidus Templeton, 1835 (Collembola, Entomobryidae): Revue d'Écologie et de Biologie du Sol, v. 24, p. 187–195.

Lane, D.J., 1991, 16S/23S rRNA sequencing, *in* Stackebrandt, E. and Goodfellow, M., eds. Nucleic Acid Techniques in Bacterial Systematics. New York: Wiley. p 115–175.

Lo, N., Casiraghi, M., Salanti, E., Bazzocchi, C., and Bandi, C., 2002, How many *Wolbachia* supergroups exist?: Molecular Biology and Evolution, v. 19, p. 341–346.

Moore, J.C., and de Ruiter, P.C. 2000, Invertebrates in detrital food webs along gradients of productivity, *in* Coleman, D.C., and Hendrix, P.F., eds., Invertebrates as Webmasters in Ecosystems. Oxford, UK, CABI Publishing, p. 161–184.

Moore, J.C., McCann, K., Setälä, H. and de Ruiter, P.C., 2003, Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics?: Ecology, v 84, p. 846–857.

Moore, J.C., Tripp, B.B., Simpson, R.T., and Coleman, D.C., 2000, Springtails in the classroom: Collembola as model organisms for inquiry-based laboratories: The American Biology Teacher, v. 62, p. 512–519.

Moore, J.C., Clarke, J.A., Heimbrook, M.E., and Mackessy, S.P., 1996, Survey of the biota and trophic interactions within Wind Cave and Jewel Cave, South Dakota. NPS report, Report to Wind Cave National Park, Hot Springs, SD 57747, USA.

Moore, J.C., de Ruiter, P.C., and Hunt, H.W., 1993, The influence of productivity on the stability of real and model ecosystems: Science, v. 261, p. 906–908.

Nation, J.L., 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy: Stain Technology, v. 58, p. 347–351.

Oksanen, L., Fretwell, S.D., Arruda, J., and Niemel, P., 1981, Exploitative ecosystems in gradients of primary productivity: The American Naturalist, v. 118, p. 240–261.

Olson, R., 1977, The hypogean ecology of Jewel Cave National Monument, Custer County, South Dakota. [Master's Thesis], Champlain-Urbana, University of Illinois.

Palmer, A.N., and Palmer, M.V., 2000, Speleogenesis of the Black Hills Maze Caves, South Dakota, U.S.A., *in* Klimchouk, A.B., Ford, D.C., Palmer, A.N., and Dreybrodt, W., eds., Speleogenesis, evolution of karst aquifers: Huntsville, AL, National Speleological Society, p.274–281.

Peck, S., 1959, A faunal survey of Wind Cave: Wind Cave National Park, South Dakota. U.S. National Park Service Report.

Roberts, A., 1974, Stability of a feasible random ecosystem: Nature, v. 251, p. 607–608.

Snider, R.J., Shaddy, J.H., and Butcher, J.W., 1969, Some laboratory techniques for rearing soil arthropods: The Michigan Entomologist, v. 1, p. 357–362.

Snider, R.M., 1973, Laboratory observations on the biology of *Folsomia candida* (Willem) (Collembola: Isotomidae): Revue d'Écologie et de Biologie du Sol., v. 10. p. 103–124.

Stam, E.M., Van de Leemkule, M.A. and Ernsting, G., 1996, Trade-offs in the life history and energy budget of the parthenogenetic collembolan *Folsomia candida* (Willem): Oecologia, v. 107, p. 283–292.

Stouthamer, R., Luck, R.F., and Hamilton, W.D., 1990, Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. Proceedings of the National Academy of Science, USA, v. 87, p. 2424–2427.

Tullberg, T., 1871, Förtecking öfver svenska podurider: öfv. K. Vetakad. Förhandl, v. 28, p.143–155.

Vanderkerchhove, T.T.M, Watteyne, S., Willems, A., Swings, J.G., Mertens, J. and Gillis, M., 1999, Phylogenetic analysis of the 16S rDNA of the cytoplasmic bacterium *Walbachia* from the novel host *Folsomia candida* (Hexapoda, Collembola) and its implications for wolbachial taxonomy, FEMS Microbial Letters, v. 180, p. 279–286.

Vandel, A., 1965, Biospeleology: The biology of cavernicolous animals: Pergamon Press, Oxford. p. 425.

Von Allmen, H., and Zettel, J., 1983, Embryonic development and postembryonic growth in a population of *Entomobrya nivalis* (Collembola): Revue d'Écologie et de Biologie du Sol, v. 20, p. 77–86.

Van Straalen, N.M., and Joose, E.N.G., 1985, Temperature responses of egg production and egg development in two species of Collembola: Pedobiologia, v. 28, p. 265–273.

Werren, J.H., Zhang, W. and Guo, L.R., 1995, Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods: Proceeding of the Royal Society of London B, v. 261, p. 55–71.

Whicker, A.D., and Detling, J.K., 1988, Modification of vegetation structure and ecosystem processes by North American grassland mammals, *in* Werger, M.J.A., During, H.J., and Van Der Aart, P.J.M., eds., Plant forms and vegetation structure: The Hague, The Netherlands, SPB Academic, p. 301–316.

Zeppelini, D., and Christiansen, K., 2003, *Arrhopalites* (Collembola: Arrhopalitidae) in U.S. caves with the description of seven new species: Journal of Cave & Karst Studies, v. 65, p. 36–42.

Zchori-Fein, E., Faktor, O., Zeidan, M., Gottlieb, Y. Czosnek, H. and Rosen, D., 1995, Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae): Insect Molecular Biology, v. 4, p. 173–178.